Biacore™ 8K+ for concentration analysis with throughput and precision

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Introduction

The measurement of protein concentration in an aqueous sample is an important assay in biochemistry research and development labs for applications ranging from enzymatic studies to providing data for biopharmaceutical lot release.

Biacore 8K and Biacore 8K+ with its eight needles opens up for assays using a parallel calibration curve as well as the serial concentration analysis. The use of a parallel calibration curve provides shorter run times, which results in data with very good precision enhanced by interchannel normalization.

This poster presents the new software features in Biacore Insight Evaluation Software that supports concentration analysis with both serial and parallel calibration curves for data generated in Biacore 8K, Biacore 8K+ and Biacore T200. In Biacore 8K and Biacore 8K+, full GxP support is offered for runs and evaluations.

Biacore 8K+

Biacore 8K+ is a high-capacity surface plasmon resonance system, efficiently delivering binding data of outstanding quality, meeting your toughest challenges in screening, characterization, process optimization, and quality control. This eight needle, high-sensitivity SPR system rapidly provides kinetics, affinity, concentration, and potency data, shortening time to results by up to eight times compared to single-needle systems.

The extended sample hotel of Biacore 8K+ holds up to 12 microplates and the buffer selector allows for use of up to 4 different buffers.

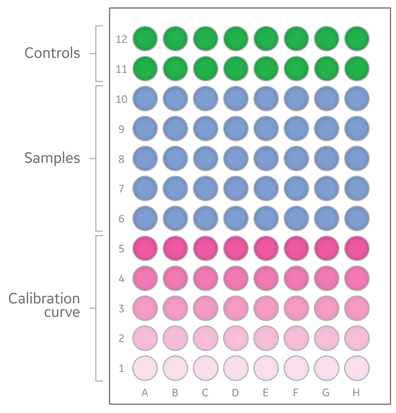
Biacore system and concentration analysis

Concentration analysis can be performed using calibration curves for each channel separately (serial approach) or with a calibration curve common for all channels (parallel approach). The parallel approach involves a channel normalization step to compensate for response differences between the channels, that is, all the responses from the calibration curve, control samples and unknown samples are adjusted.

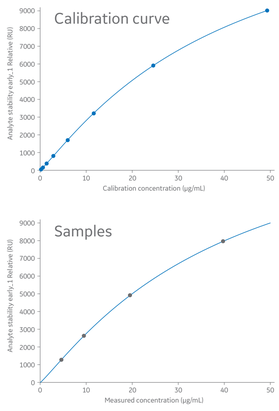
Serial concentration analysis

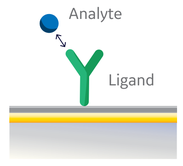
- Providing data with excellent precision
- Each channel holds its own calibration curve
- Beneficial when analyzing a large number of samples and/or high precision is required

Experimental setup



Results channel 1 (example)



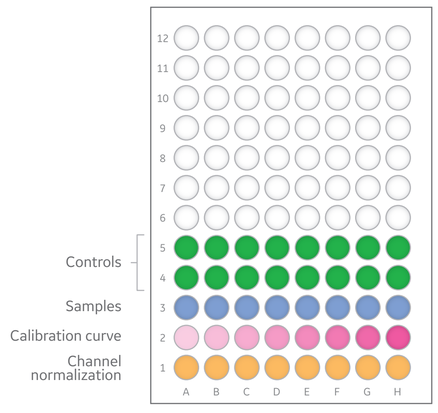


Direct binding assay

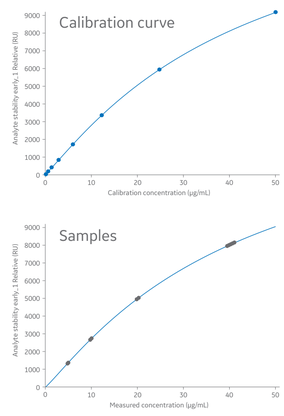
Parallel concentration analysis

- Providing data with very good precision
- Calibration curve across channels, only one cycle needed for calibration curve
- Shorter run time than serial concentration analysis
- Channel normalization accounts for differences in response from the used channels

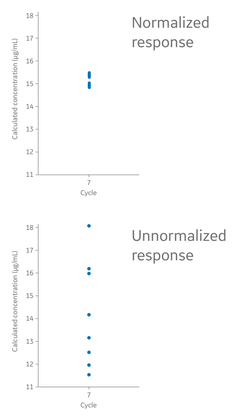
Experimental setup



Results channel 1-8 (example)



Channel normalization



The ligand used in the experiments was protein A (Sensor Chip Protein A, GE Healthcare) and the analyte was human IgG

Application example

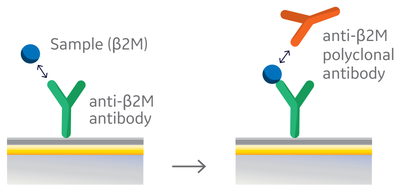
A concentration analysis to quantitate β2-microglobulin (β2M) in human plasma samples was performed using Biacore 8K+ set up with a parallel calibration curve. Comparison with an ELISA was made using identical sample and standard.

Experimental setup

Workflow overview 96-well plate with 40 samples (in duplicate)

Biacore assay

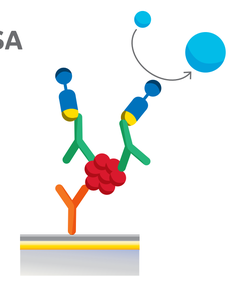
An enhancement molecule (anti-β2M polyclonal antibody) was included to increase the specificity of analyte binding due to issues with nonspecific binding from the plasma samples to the ligand (anti-β2M antibody). However, nonspecific binding to the dextran matrix was ruled out in a separate control experiment (not shown).



1	Dock a new sensor chip and immobilize
2	Prepare all reagents, samples and standard
3	Add all reagents, standard and sample to wells according to the plate layout in the predefined Biacore method. Cover and start the Biacore run.
4	Evaluate result

Assay preparation 2 h incl. surface preparation/coating.
Total assay time: 3.25 h
Total time to results: 5.25 h

ELISA



1	Prepare all reagents, samples and standard
2	Add standard and sample to wells. Cover and incubate at RT for **2.5 h**
3	Wash plate four times
4	Add biotinylated Ab to wells. Cover and incubate at RT for **1 h**
5	Wash plate four times
6	Add Streptavidin-horseradish peroxidase (HRP) reagent to each well. Cover and incubate at RT for **45 min**
7	Wash plate four times
8	Add tetramethylbenzidine (TMB) to each well
9	Develop plate at room temperature in dark for **30 min**
10	Add stop solution to each well
11	Measure absorbance and read results

Assay preparation 2 h excl. microplate coating.
Total assay time: Less than 5.5 h
Total time to results: 7.5 h (excl. microplate coating)

Benefits of Biacore analysis compared to ELISA

- Automated real-time determination of active concentrations with increased precision
- Saves on development time—no labeling of secondary reagents and enhancements might not be needed
- Easier sample preparation and ability to queue several methods and runs in sequence
- Reduced labor intensity—higher degree of automation and all hands-on activities performed in one step
- Accurate quantitation and/or affinity analysis of low-affinity/high K_D analytes often missed by ELISA

Conclusions

- Biacore 8K and Biacore 8K+ offer tools that allow for efficient concentration determination
- Biacore Insight Evaluation Software Concentration and Potency Extension package supports concentration analysis with both serial and parallel calibration curves
- The precision of concentration analysis using the parallel calibration curve is enhanced by channel normalization
- Concentration analysis assays performed in Biacore 8K, Biacore 8K+, and Biacore T200 can easily be evaluated in Biacore Insight Evaluation Software with the extension package
- The large hotel of Biacore 8K+ combined with the concentration analysis functionality enables a higher degree of automation compared to ELISA

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